quantities of ascorbate and tartaric acid for the analyses. However, since the weight of reductant varies slightly, the reagent must be free of nitrite or other compounds yielding nitric oxide. Ascorbate gave no nitric oxide signal but commercial samples of reagent grade potassium iodide gave a background signal corresponding to 300 ppb sodium nitrite. This high background was considered unsuitable for the determination of low levels of nitrite.

The analyses of an acidified 100 ppb sodium nitrite solution using ascorbate as a reductant resulted in a signal 7 times that without ascorbate. Without tartaric acid present, no signal greater than background was generated. Walters et al. (*4*) and Cox (*7*) used acetic acid for acidification, which required the use of alkali traps to remove the volatile acid before passing the nitric oxide gas through the detector. In order to eliminate the traps, we selected tartaric acid since it is nonvolatile and has an appropriate pK_a (2.96).

Linearity, Sensitivity, and Precision. The nitric oxide response of the instrument was linear over a range equivalent to 2.5–2000 ppb sodium nitrite in a 2-mL sample. The calibration equation in arbitrary area units (au) is

$$\text{au} = 4.63 \times \text{ppb} + 10.7 \qquad r = 0.9998 \qquad s_{yx} = 67.73 \tag{1}$$

Sample volume in the reactor was varied up to 10 mL with no variation in the measured nitric oxide for a given quantity of nitrite thereby demonstrating that the method is insensitive to water. The detection limit, assuming a 2:1 signal to background ratio, was 25 ng of sodium nitrite, equivalent to 25 ng/10 mL or 2.5 ppb, compared to a sensitivity of ca. 250 ppb for Griess analysis. For the determination of instrument repeatability, 500 ppb $NaNO_2$ standard solutions were measured in duplicate over a period of several weeks. The pooled standard deviation (s) value for replicates was 7.62 au (CV = 5.5%) for values ranging from 116 to 166 au. The average of all standards was 141 au (s of 13.9 au). Since the latter value was higher than the replicate s value, the chemiluminescence detector was calibrated daily with a nitrite standard. The precision of the method was determined by duplicate analysis of eight meat samples prepared as described containing varying amounts of nitrite. The pooled s value was 6.3 ppm for an average of 96.2 ppm (CV = 6.5%) for the 16 (8 × 2) nitrite-containing meat samples.

Interfering Compounds. The addition of 1700 ppm sodium nitrate or 100 ppm sodium sulfite gave no nitric oxide response and had no adverse effect on sodium nitrite measured in a 500 ppb sodium nitrite solution. One part per million butylnitrite and 1 ppm *N*-nitrosodimethylamine produced responses of 8.3 and 2.5%, respectively, of that of an equimolar concentration of sodium nitrite. *N*-Nitrosodimethylamine showed no response at the 10-ppb level; therefore, its presence in foods would not interfere significantly with the nitrite measured. Alkyl nitrites or unstable nitrosothiols, if present in significant amounts, could lead to a measureable error.

Comparison of Detection Methods. The results obtained by chemiluminescence, Griess colorimetric, and differential pulse polarographic measurements of nitrite in meat slurries are shown in Table I. A loss of nitrite occurred in all the samples because of the reaction of nitrite with endogenous components and/or added reductants. Therefore, the testing of these methods takes place under this condition. The sodium nitrite values were comparable for all three methods in samples without an added reductant, whether they were treated with charcoal or not. The CLD method yielded the highest nitrite values when a reductant was added; however, after charcoal treatment the values were equivalent. Clearly, the lower nitrite values obtained by direct measurement, with 550 and 2200 ppm ascorbate added, indicated interference of the reductant with the Griess and DPP methods. In these two methods, nitrite is measured indirectly from a nitrosated product that involves competitive reactions. In the Griess reaction sulfanilamide is nitrosated to form an azo compound which is coupled with 1-napthylamine to yield a pink dye. In DPP, diphenylamine is nitrosated in the presence of a catalyst, thiocyanate ion, to form the corresponding nitrosamine. Ascorbate and to a lesser extent other reductants compete with the aromatic amines for the nitrosating species (N_2O_3), thereby yielding low results when nitrite and reductants are simultaneously present. The addition of charcoal as recommended by Adriaanse and Robbers (*10*) removed the ascorbate, whereupon the nitrite values measured by the Griess and DPP methods were equivalent to those measured by the CLD. The sulfhydryl group of the added cysteine caused further loss of nitrite (Table I) as expected (*9*), but the measured concentration of nitrite was the same for the CLD and Griess method even after charcoal treatment. The lower values for thee cysteine-containing samples were not due to interference, since cysteine was found by separate experiments not to affect the nitrite measured by CLD in standard solutions. There was, however, an interference by sulfhydryl groups in the DPP determination, which appeared to be partly eliminated by the charcoal treatment.

Table I. Determination of Residual Nitrite in Meat-Derived Samples with and without Charcoal Treatment

added[a]		amt of sodium nitrite, ppm					
		direct			charcoal		
salt, %	reductant, ppm	CLD	Griess	DPP	CLD	Griess	DPP
0	0	104	110	107	104	101	110
0	550 ascorbate	101	55	30	91	92	90
0	2200 ascorbate	80	18	16	82	77	80
0	2400 cysteine	86	79	54	81	80	70
5.9	0	110	106	104	100	102	100
5.9	550 ascorbate	64	35	11	62	64	60
5.9	2200 ascorbate	45	9.7	4.2	44	45	40
5.9	2400 cysteine	75	69	60	68	69	60

[a] 140 ppm $NaNO_2$.

Sodium chloride alone does not affect nitrite analysis by any of the three methods in the absence of a reductant. In the presence of a reductant, salt causes a greater loss of nitrite as measured by all three methods but does not affect the reductant interference in the Griess and DPP methods.

Accuracy. When the nitrite was measured under the best conditions, i.e., after elimination of the known interference by charcoal addition, the nitrite values for all three methods of measurement were within the limits of precision (±6.5%) from their mean. Since the measurements were made on samples from which the known interference, ascorbic acid, was removed (*13*), it appears that the maximal amount of free nitrite was being measured.

Since no sample preparation procedures are required, the analysis time for this nitrite method is approximately 10 min for a triplicate measurement. The accuracy and freedom from known interferences recommend it as a reference technique not only for cured meats but also for other samples of biological origin.

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Determination of Nitrite in Cured Meats by Chemiluminescence Detection

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Methods for determining nitrite in foods and other biological systems are of considerable interest because nitrite is an important precursor in nitrosamine formation. An excellent review of a variety of nitrite methods has been published by Usher and Telling (*1*). While chemiluminescence detection (CLD) has been used for the measurement of nitrogen oxides in air (*2*) and adapted for use as a specific detector for nitrosamines (*3*), it has been utilized little for nitrite analysis.

Walters et al. (*4*) have developed a procedure for determining nitrite concentrations in foods by acidifying the sample with acetic acid and measuring the nitric oxide evolved with a chemiluminescence detector. While it is not immediately evident that the technique is not subject to the same interferences as other methods, it does have the potential of being at least 1 order of magnitude more sensitive than the colorimetric methods in use today. Their method has some disadvantages, however. Water was found to decrease the response of the method, thereby limiting its applicability to food products. Drying procedures are cumbersome, and nitrite could be lost during drying. Walter's method involves only acidification of the sample, thereby relying upon acid dismutation reactions to produce nitric oxide. However, nitrous acid decomposes by mono-, bi-, and trimolecular reactions to form a number of nitrosating species, including NO^+ as well as nitric oxide (*5*, *6*). The amount of nitric oxide that can be produced is dependent upon a balance among competing reactions that can be altered by various compounds present in food. For example, nitrosatable compounds, such as primary and secondary amines and reductants, could react with the nitrosonium ion NO^+, thereby yielding less nitric oxide and consequently low nitrite values. A more homogeneous reaction would be desirable, since the measured compound is nitric oxide. Cox (*7*), however, has resolved this problem by driving the nitrous acid decomposition reaction to nitric oxide by adding sodium iodide as a reductant. We have further investigated the addition of reductants and other acidulants and have developed a procedure that is an improvement upon the previous techniques.

To assess the effectiveness of the chemiluminescence procedure for determining nitrite concentrations, we compared it with the Griess colorimetric procedure and differential pulse polarographic method (*8*), using as a test medium a meat slurry containing either sodium ascorbate or cysteine, both of which either cause loss of nitrite in meat (*9*) or interfere

in the analyses (*8*, *10*). We added charcoal, which specifically eliminates the ascorbate interference (*10*), to the test system to compare the effectiveness of the three analytical procedures, free of known interferences.

EXPERIMENTAL SECTION

Reagents. All chemicals were reagent grade or highest purity available and were used without further purification.

Test Media. The technique was tested both in standard nitrite solutions and in meat samples in which a loss of nitrite had occcurred. The latter were prepared from pork skeletal muscle slurries (meat:water, 1:2) containing 140 ppm (2 mM) sodium nitrite. Eight individual samples were produced with and without 5.9% sodium chloride and with either no added reductants, 550 ppm or 2200 ppm ascorbate, or 2400 ppm cysteine. These eight test slurries were prepared by blending the meat with an equal weight of 420 ppm (6 mM) sodium nitrite. The resulting slurry (210 ppm) was divided into four equal portions, and half the volume of water or solutions of the appropriate concentrations of ascorbate or cysteine were added to give the final desired concentrations. Each of these samples was again divided, and solid sodium chloride was added to one portion to give the final desired concentration of salt. The slurries were heated at 70 °C for 1 h and then stored at 4 °C for 1 week to achieve a measurable and significant loss of nitrite. The samples were analyzed for nitrite by direct measurement of nitrite in the supernatant of the centrifuged slurry and after addition of 1 part Darco nitrite-free charcoal to 100 parts of a 1:10 dilution of the slurry as recommended by Adriaanse and Robbers (*10*). The charcoal samples were shaken for 30 min and centrifuged, and the supernatants were then analyzed for nitrite.

Griess. Measured portions of the clear centrifuged supernatants from the two preparation methods were added to 1 mL of the Griess reagent (10.0 mM sulfanilamide and 2.0 mM 1-naphthylamine) and diluted to 10 mL. Portions were 0.1 mL of the untreated sample supernatant or 1.0 mL of the supernatant after charcoal treatment. After $^1/_2$ h, the concentration was determined from the absorbance at 515 nm, $a = 1.65$ ppm^{-1} cm^{-1}, by a Cary 14 spectrophotometer.

Caution: 1-Naphthylamine has been designated by OSHA as a Class One carcinogen (*11*). Therefore, all proper precautions for its use were observed during this study.

Differential Pulse Polarography (DPP). Nitrite determination followed the procedure of Chang et al. (*8*). To 7.5 mL of sample was added 2.5 mL of supporting electrolyte (10 mL of 0.01 M KSCN, 10 mL 0.4 M perchloric acid, and 5 mL of 2.6×10^{-3} M diphenylamine in 40% methanol), and the nitrite was determined as diphenylnitrosamine by scanning from −0.34 to −0.8 V at a rate of 2 mV/s with a mercury dropping electrode set at 1 drop/s. Nitrite concentration was calculated by comparing peak heights of the sample to those of a standard nitrite solution. Measurements were made with a Model 364 polarographic analyzer and Model 310 polarographic detector (Princeton Applied Research, Princeton, NJ).

Chemiluminescence Detection. The centrifuged samples were diluted 1:200 with distilled water prior to analysis. Peak areas were measured by the integrator supplied with the Thermal Energy Analyzer (Model 502, Thermo Electron Corp., Waltham, MA). Concentrations were calculated by comparing sample peak areas to that of a standard nitrite solution. Standards were measured several times a day to check repeatability. A minimum of three injections was run on each sample to determine the mean value.

Apparatus and Operating Procedure. Walters et al. (*4*) and Cox (*7*) swept the reaction flask with an inert gas in order to measure the total amount of nitric oxide produced. We chose to measure the evolved nitric oxide by injecting small portions into the chemiluminescence detector by means of an evacuated sample loop and gas sampling valve. Our technique uses a simpler gas regulation system and permits multiple nitric oxide measurements, thus reducing the possibility of errors that might go undetected with a single measurement. A schematic diagram of the sampling system interfaced with the Thermal Energy Analyzer is shown in Figure 1.

A 100-mL, three-neck, round-bottom flask containing a magnetic stirring bar was used as a reactor vessel. One neck was

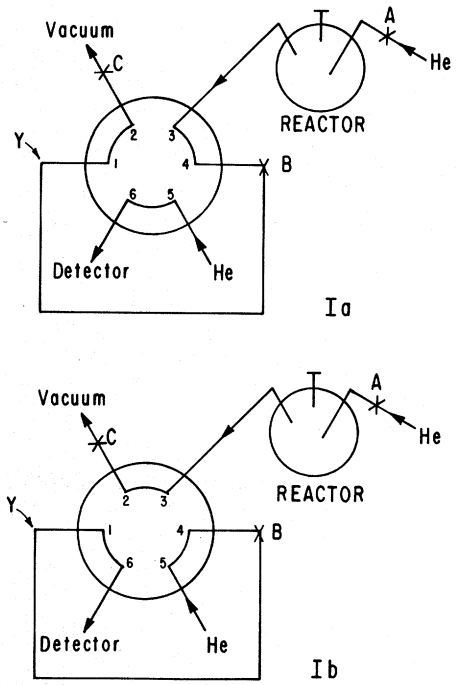

Figure 1. Schematic representation of sampling system for nitrite analysis: (a) load position; (b) inject position.

connected through toggle valve A to a helium source for flushing purposes and another neck to port 3 of a Carle No. 5518 six-port minivalve. The sample loop was a Teflon tube of approximately 8 mL internal volume connected to the sample valve at ports 1 and 4, the latter having valve B attached. Port 2 with toggle valve C was used for evacuating the sample loop. Port 6 was connected to the direct inject port of the thermal energy analyzer. The helium supply to the direct inject furnance was internally disconnected and attached to port 5 of the gas valve.

A typical measurement was conducted as follows: Approximately 56 mg of sodium ascorbate (NaAsc) and 44 mg of tartaric acid were placed in the bottom of the 100-mL three-neck, round-bottom reactor flask. In the load position (Figure 1a) with valves A and B open and the loop disconnected from the valve at point Y, the entire system was flushed with helium to remove air. Then valves A and B were closed, the loop was reconnected at point Y, and the system was evacuated up to valve B by opening valve C, which was connected to a vacuum pump. We used the pump in the thermal energy analyzer as our source of vacuum by connecting it to a TEE joint inserted between the direct inject furnace and the mode select valve. The nitrite-containing solution was injected into the reactor by syringe through a gas tight serum cap covering the center neck of the three-neck flask. The reaction mixture was stirred continuously by a magnetic stirrer. Valve C was closed and valve B was opened. To introduce the evolved nitric oxide into the detector, after 10 s, the gas sampling valve was rotated from the load to the inject position, Figure 1b. Repetitive injections were made by merely rotating the gas valve back to the load position for 10 s and then to inject. The concentration of nitric oxide was then determined by chemiluminescence.

RESULTS AND DISCUSSION

Effect of Ascorbate and Tartaric Acid on Nitric Oxide Production. We chose ascorbic acid as the reductant because under mildy acid conditions nitric oxide is the only product of the reduction (*12*). A preliminary series of nitrite determinations showed that the nitric oxide response by the CLD was unaffected by amounts of sodium ascorbate from 14 to 220 mg and tartaric acid from 11 to 180 mg. With 140 ppm (2 mM) sodium nitrite, the lowest of the above values corresponds to a millimolar ratio of about 18:18:1 (ascorbate:tartaric acid:nitrite). Since the reactants were in such excess, weighing the reagents was not necessary when running the analyses on a routine basis. A 2.0×0.4 cm spatula contained sufficient